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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
We perform modelling of macromolecular systems by a combination of computational methods including Molecular Dynamics, Langevin Dynamics, and Monte Carlo, and theories including statistical mechanics and field theories. Our modelling work is conducted synergistically in collaboration with many experimentalists participating in the AFOSR program of "Natural Materials, Systems & Extremophiles".

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## Abstract:

We have performed modelling and theory of spontaneous self-assembly of biomimetic systems and directed growth of crystals under the influence of polypeptide sequences. The various macromolecular systems in this project were treated by a combination of computational methods including Molecular Dynamics, Langevin Dynamics, and Monte Carlo, and theories including statistical mechanics and field theories. Specifically, we have investigated biomineralization, S-layer growth, and virus assembly. In the area of biomineralization, we focused on guiding the growth direction of zinc oxide crystals by specific sequences of polypeptides. We have identified a major paradigm for spontaneously selecting a particular morphology of a biomimetic crystal. We have developed a set of design rules for the control of mineral morphology in terms of the sequences of the polypeptide. The basic feature of these rules may be paraphrased as as follows: given a set of competing growth sectors, the polymer sequence that adsorbs the most to a particular growth front, promotes all other growth sectors, independent of their intrinsic growth rates. By poisoning the crystal face of a sector that is growing spontaneously very fast relative to other sectors, the slower growth sectors can be promoted.

## Objectives:

The major goal of the project is to develop theoretical models and computational algorithms to model the phenomenon of biomimeralization. More specifically, the goal is to model the thousands of potential sequences of polypeptides used in fabricating controlled morphologies of zinc oxide crystals and silica particles, and then to identify the particular sequences that are most suited for the desired purposes.

In the first year, the goal was to build a multi-scale modeling engine and bring Molecular Dynamics, Brownian Dynamics and Monte Carlo algorithms together. Specific system to be explored was zinc oxide growth. Since the growth medium contains charged ions, the role of electrostatics has to be modeled accurately.

In the second year, the goal was to model different polypeptide sequences near the different crystallographic planes and find the correlation between the polypeptide sequences and response of zinc oxide growth.

In the third year, the goal was to make predictions in terms of what polypeptide sequences will lead to spontaneous selection of desired crystal morphologies, and validate the modeling results with the experimental results from the other teams in the program.

## Findings:

First, we prepared the various crystallographic planes, based on the ZnO crystal lattice parameters. We modeled the interaction between a polypeptide of a prescribed sequence, by accounting for all electrostatic interaction between the ions and explicit counterions, as sketched in Figure 1.

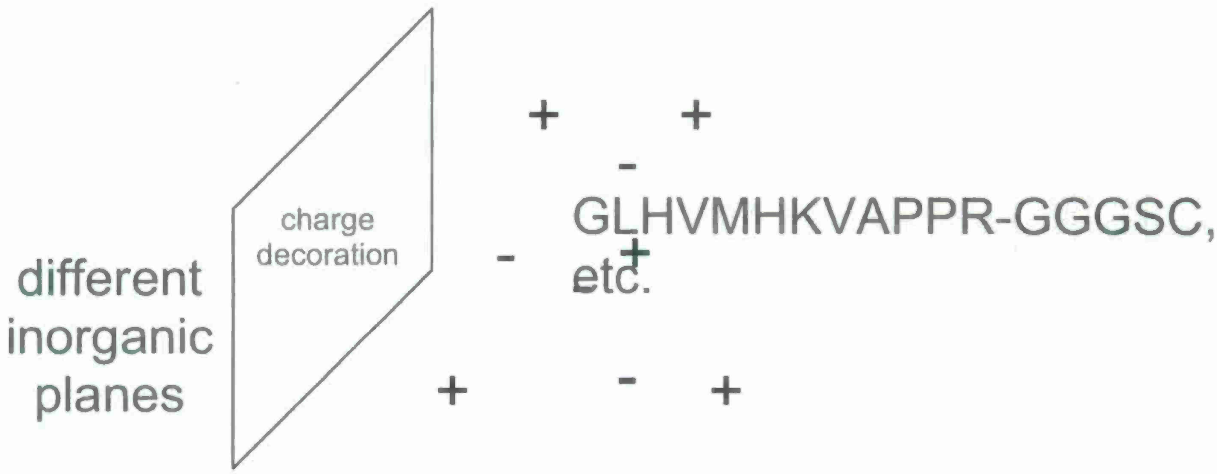


Figure 1: A polypeptide with explicit counterions in front of a growth front. The charge decoration on the growth front depends on the particular crystallographic plane.

A typical simulation system is given in Figure 2. One surface is charge-decorated and

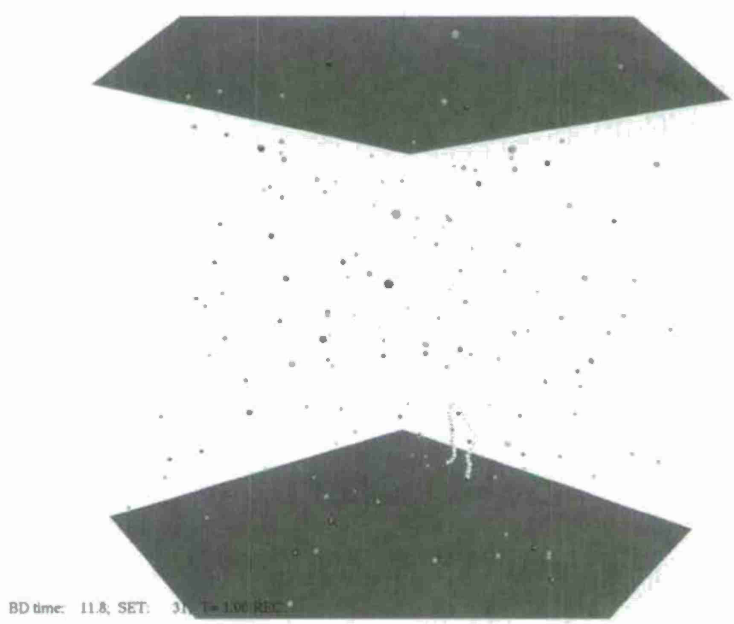


Figure 2: Brownian Dynamics of a polypeptide and counterions near an interface.

the other surface is neutral to confine the polypeptide within a simulation box. We computed the electrical potential put out by the interface into the liquid phase. In an effort to check and calibrate the computational procedure, we compared the simulated results with the analytical theory of Gouy-Chapman for planar interfaces and the agreement was good as shown in Figure 3.

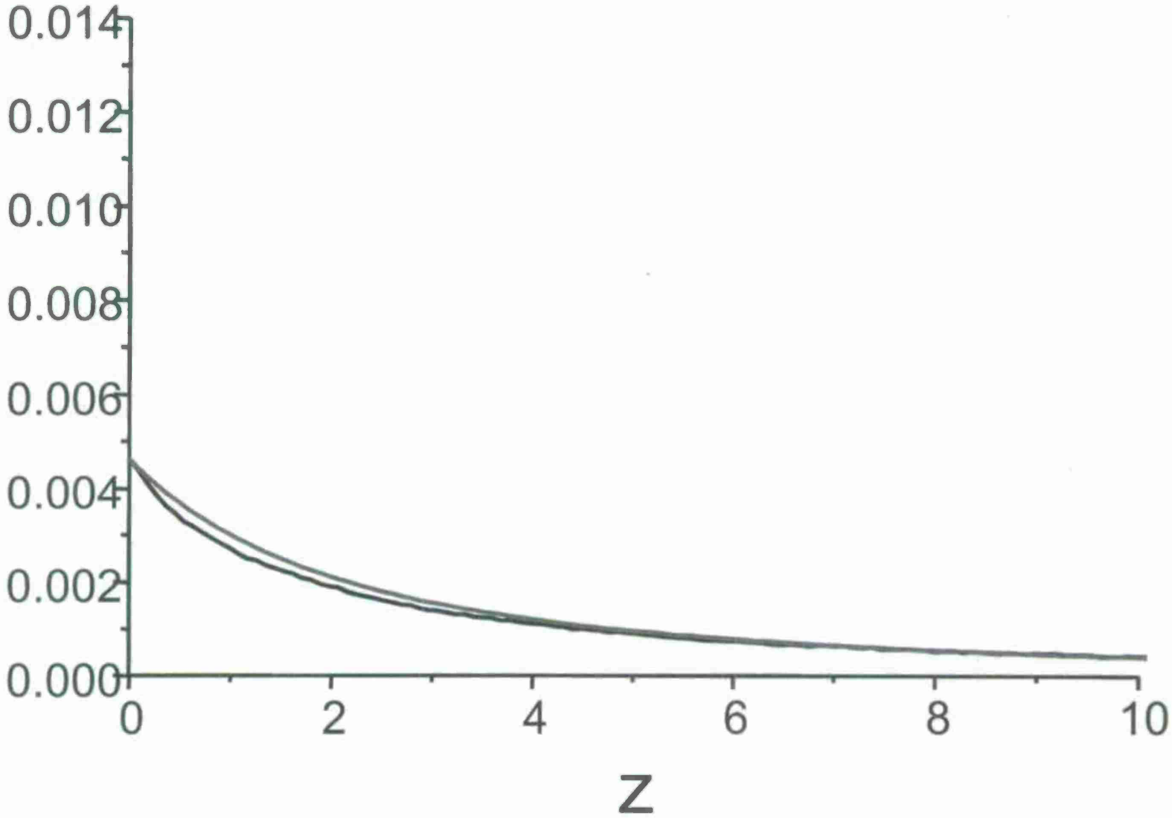


Figure 3: Plot of charge density as a function of the distance from the interface. Black curve is the result of simulations and the red curve is the classical Gouy-Chapman theory. The surface charge density is 0.024. This comparison validates the simulation program.

A series of simulations were carried out based on this approach. After conducting thousands of trajectories for tens of specified polypeptide sequences, we found out that we needed to be more clever in order to decode the role of sequences on particular mineral interfaces. After pondering over, namely thinking deeply, instead of generating terabytes of just data, we recognized that there is a special interaction going on between the polypeptide sequences and zinc oxide surfaces. To be brief, we recognized that if the polypeptide sequence has the capacity to form zinc finger-like motif, then those sequences would preferentially bind to a surface that is rich in zinc content. Our initial conjecture was then supported by sporadic data in the literature that histidine and cystein amino acid residues bind strongly to 002 planes of zinc oxide. This recognition led to a major breakthrough in terms of our understanding of morphology selection in zinc oxide. Our hypothesis was confirmed by detailed simulations. For example, the polypeptide sequence mentioned in Figure 1 would strongly adsorb to 002 plane (Figure 4a), which is high in the density of zinc; on the other hand 100 plane or 101 plane would not bind the polypeptide (Figure 4b).

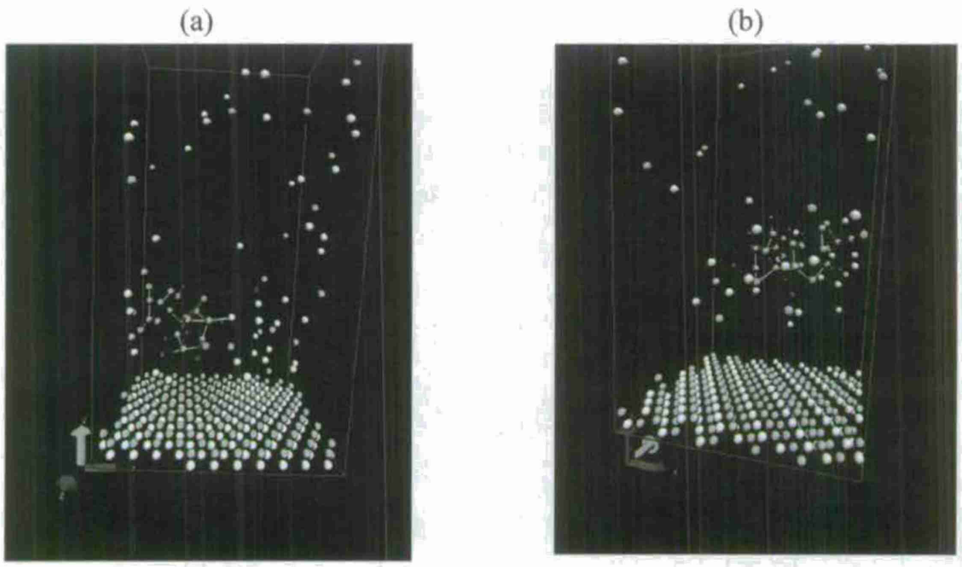

Figure 4: Binding of the polypeptide sequence of Figure 1 to (a) 002 plane of zinc oxide and (b) 101 plane.

In performing these simulations we developed coarse-grained potentials based on mult-scale modeling, as sketched in the scheme below (Figure 5). We have used a combination of statistical mechanics theory and computer modeling. Our theory combines our earlier theories of polymer adsorption and nucleation in crystallization. Our multi-scale modeling uses a combination of Molecular Dynamics, Brownian Dynamics and Monte Carlo to explore the length and time scales over eight orders of magnitude. The scheme is illustrated in the chart below. One of the unique strengths of our approach is the capability to implement theoretical approaches along with simulation methods. It must be mentioned that quantum effects and chemical reactions at the interfaces are neglected in our computer modeling so far.

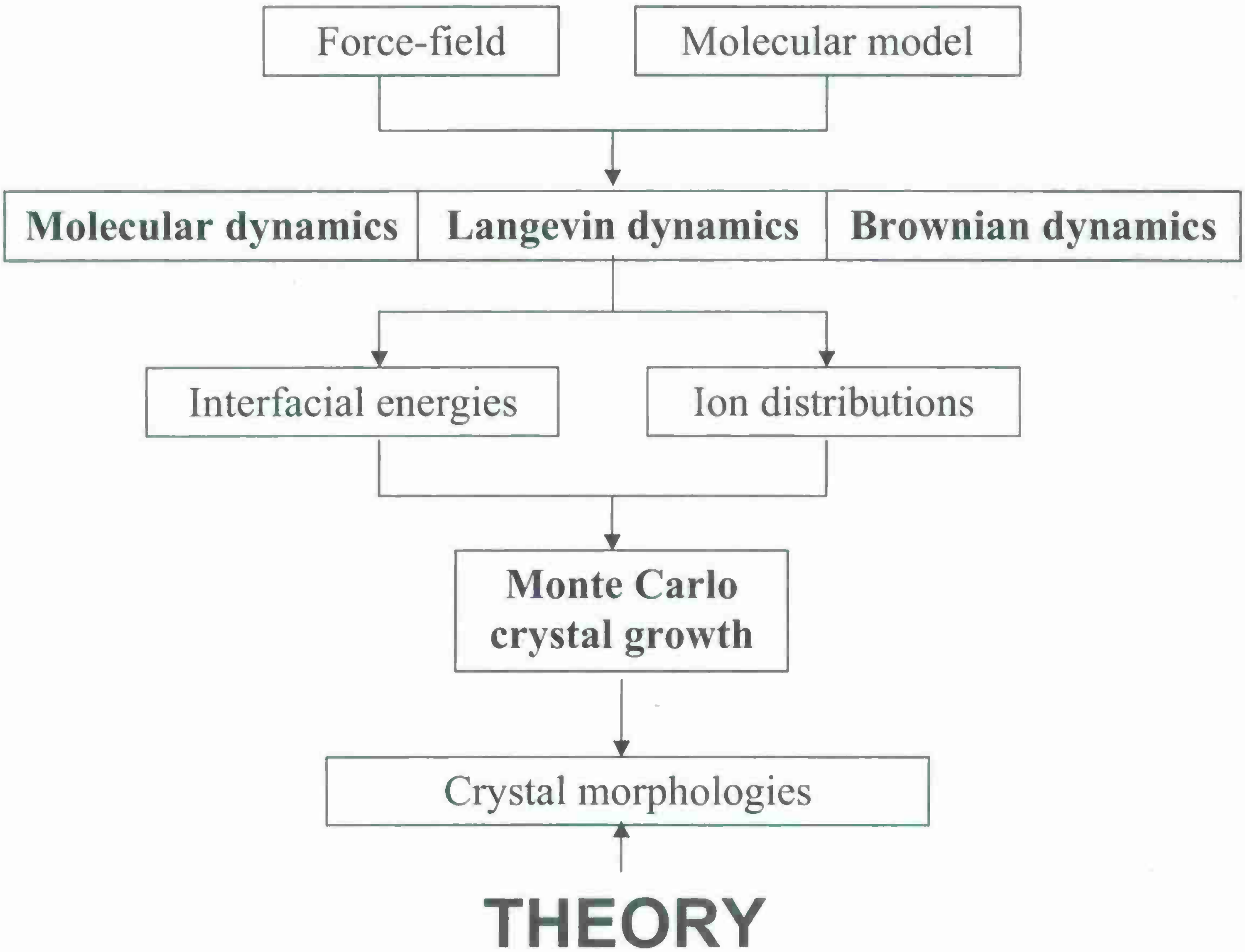


Figure 5: Our scheme of multi-scale modeling used in simulating the interaction between polypeptides and inorganic interfaces.

We have performed hundreds of trajectories for each of the candidate sequences. The number of sequences that we explored was about five hundred with twenty amino acid residues for each polypeptide chain. We also recognized that it is essential to account for the presence of water explicitly (Figure 6).

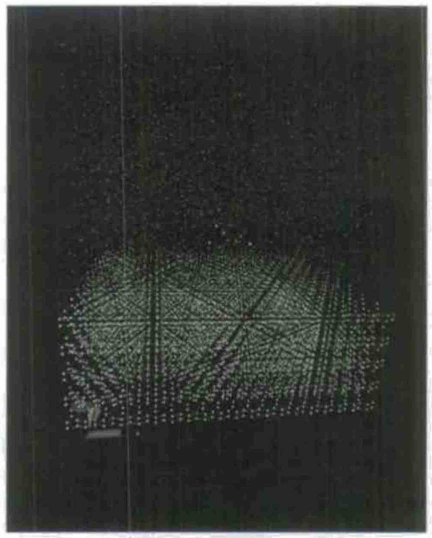

Figure 6: In the later versions of our modeling, we have taken water explicitly.

While too rich data were pouring out of these simulations, we decided to seek more fundamental underlying themes behind biomineralization than surrendering to computers and trial-and-error based experimental measurements. Specifically, we were aware of the discovery made in the laboratory of R. Naik (Wright Patterson) about the selection of morphology by the presence of polypeptides (Figure 7).

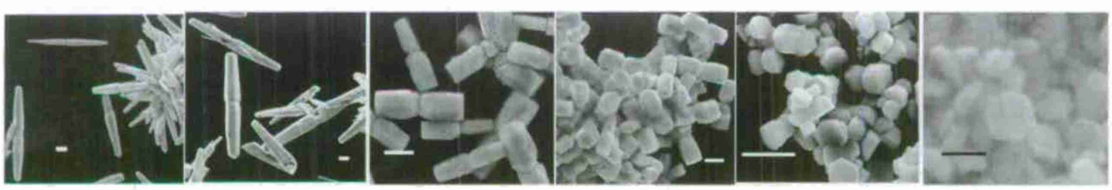

Figure 7: In the panels from left to right, the concentration of the polypeptide (GLHVMHKVAPPR-GGGC) increases. Other sequences, for example, TVSRPTAPYVTP, has no effect on the selection of morphology. (The data are from R. Naik's group.)

Based on our simulation results for various polypeptide sequences at 002, 101, 100 crystal planes, we recognized that the presence of a combination of a pair of histidines and cysteins in the motif of zinc fingers is critically needed to poison the 002 plane. If this were to happen, then the faces of the needles would not grow and only the lateral growth, as a disk, would be allowed. Therefore, we decided to parametrize the specific interaction between the polypeptide and the inorganic surface as an interfacial energy and investigate the consequences of the polypeptide concentration-dependent interfacial energy on the spontaneous selection of morphology. We then compared the surface coverage of the polypeptide, which depends on the polypeptide concentration, with the aspect ratio of the morphology. We were able to recover the experimental results on morphology control. This success led to the promulgation of a general theory of spontaneous selection of polypeptide-mediated crystal morphology.

The idea was to consider a crystal nucleus of a particular shape, say a tablet-shape, as illustrated in Figure 8. Then we wrote the free energy of this tablet in terms of the bulk free energy and the various interfacial energies. Then, we constructed the classical nucleation theory in combination with polymer adsorption at the growth interfaces.

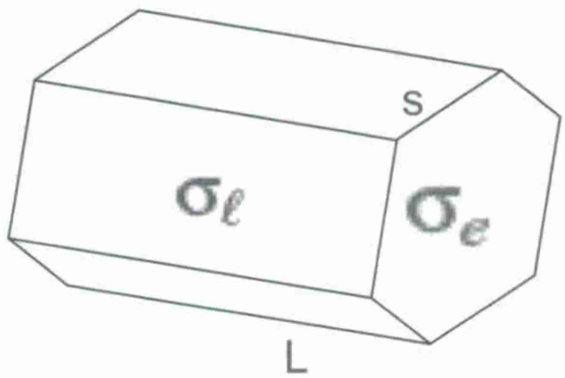


Figure 8: A tablet with length L and side s.

In the new theory developed by us, the polymer molecules adsorb to the growth surfaces and intrinsically modify their interfacial free energies. Given the constraints of the interfacial energies, and the gain in free energy associated with the formation of the stable mineral, the equilibrium aspect ratio of the crystal and the growth kinetics can be derived. For example, the aspect ratio, s/L, is dictated by ratio end-surface energy to the lateral surface energy:

$$\frac{L}{s} = \sqrt{3}\frac{\sigma_e}{\sigma_\ell}$$

The comparison of our prediction and the experiments is given in Figure 9. The agreement is remarkable, vouching for the applicability of our new paradigm of adsorption-nucleation mechanism of biomineralization.

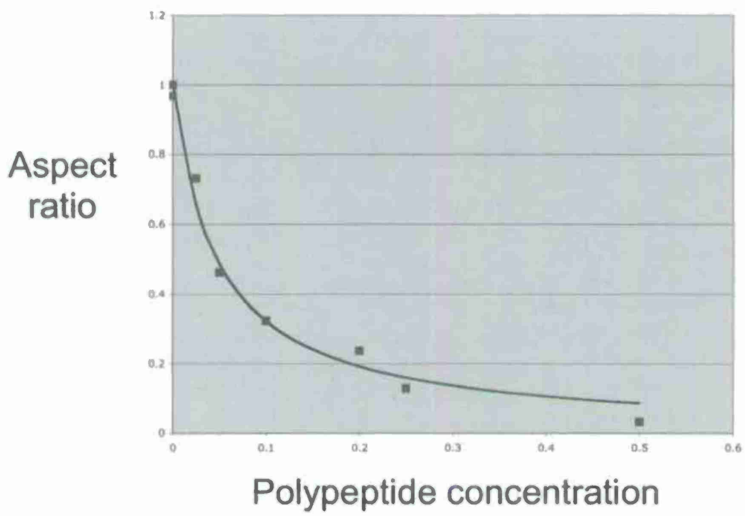


Figure 9: The curve is predicted by our theory and the squares are the data from R. Naik's laboratory.

In terms of the above-mentioned interfacial energies, the following growth kinetics has been derived analytically. The prediction is that disks, favored at higher polypeptide concentration, grow faster than the needles, and vice versa. In Figure 10, $\Delta\mu$ is the supersaturation in zinc oxide, and a is the spacing between two crystallographic planes. $k_B T$ is the Boltzmann constant times the absolute temperature.

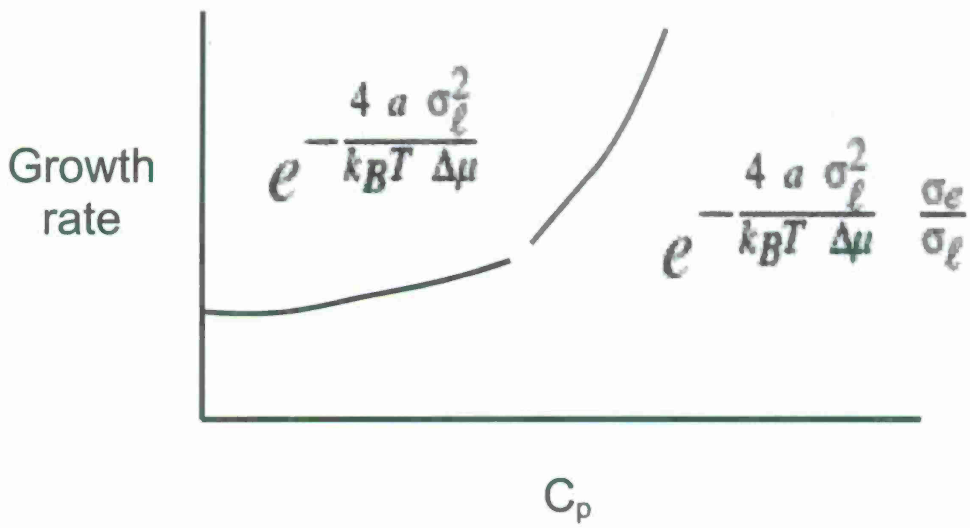


Figure 10: Predicted growth kinetics as a function of polypeptide concentration. The regime on the left corresponds to growth of the needles and the one on right to disks.

In addition to biomineralization modeling, we have performed multi-scale modeling for the assembly of ss-RNA/DNA viruses, as an attempt to compare with templating with an organic molecule instead of inorganics. As an example, we considered the assembly of Parovirus (Figure 11). Our simulations clearly demonstrate that the virus assembly is a nucleation-growth process, and the kinetics of assembly can be controlled by the sequences of the proteins and polynucleotides in a predictive manner.

(a) (b)

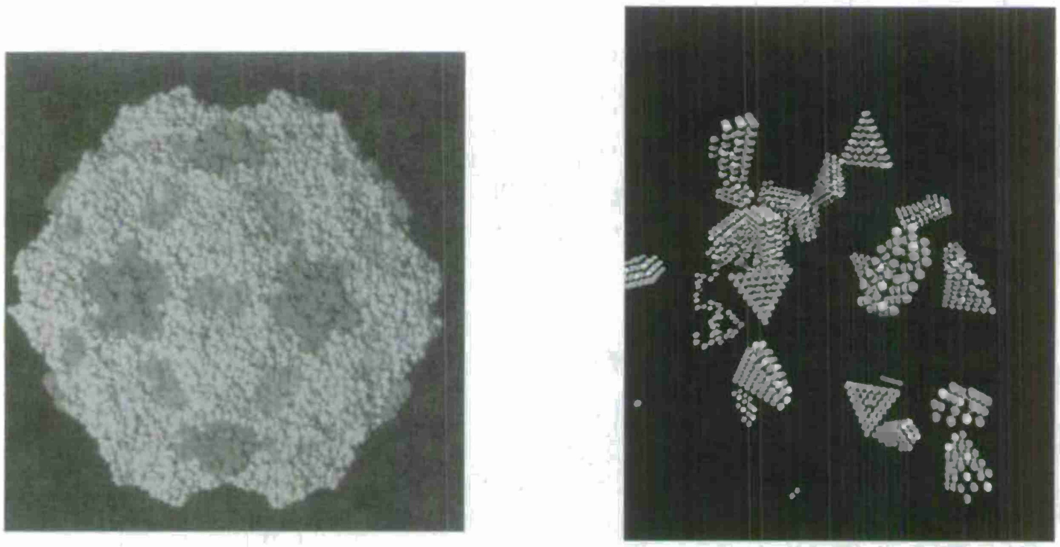

Figure 11: Multi-scale modeling of Parovirus. (a) Three-dimensional reconstruction. (b) Coarse-grained simulation of the assembly process.

## Supported Personnel:

1. Mark Kelly (Graduate student)
2. J. P. Mahalik (Graduate student)
3. Chris Forrey (Graduate student)
4. Christian Ruthard (Graduate student)
5. Ajay Panwar (Postdoc)
6. M. Muthukumar (PI)

## Collaborations:

1. Rajesh Naik: We collaborated extensively on the navigation of morphology of zinc oxide in the presence of polypeptides.
2. Carol Perry: We had extensive discussions on the modeling of silica network and biomineralization with silica. He group has used our computational method.
3. Paul Trulove: We preformed dynamic light scattering measurements on ionic liquids containing polymers.

4. Jin Montclare: We performed extensive dynamic light scattering and static light scattering on diblock and triblock copolymers containing coiled coil and elastin moieties.
5. David Kaplan: We have calculated morphology phase diagrams for silk-containing block copolymers.
6. Dietmar Pum and Uwe Sleytr: We have performed multi-scale modeling of S-layer proteins and their assembly.

## Publications:

(i) M. Muthukumar, "Competitive adsorption-nucleation in polypeptide-mediated biomineralization," *J. Chem. Phys.*. **130**, 161101 (2009).

(ii) C.Forrey and M. Muthukumar, "Electrostatics of capsid-induced viral RNA organization," *J. Chem. Phys.* **131**, 105101 (2009).

(iii) J. Zhang and M. Muthukumar, "Simulation of nucleation and elongation of amyloid fibrils", *J. Chem. Phys.* **130**, 035102 (2009).

(iv) J. S. Haghpanah, C. Yuvienco, D. E. Civay, H. Barra, P.J. Baker, S. Khapli, N. Voloshchuk, S.K. Gunasekar, M. Muthukumar, and J.K. Montclare, "Artificial protein block copolymers: Blocks comprsing two distinct self-assembling domains", *ChemBioChem*, **10**, 2723 (2009).

## Interactions/Transitions:

The development of the new paradigm is a shift in the way the process of biomineralization has been thought about so far. The couching of the biomineralization phenomenon as a competitive "adsorption-nucleation" process unifies the diverse sets of these processes, and places the critical element of the challenge on the interfacial interaction between the polypeptides and the inorganic interface.

In fact, our work entitled "Theory of competitive adsorption-nucleation in polypeptide-mediated biomineralization" was selected as one of the Editors' Choice for 2009 in the Journal of Chemical Physics. Our predictions on the kinetics of zinc oxide growth and analogous predictions on the growth of ice, are being tested in the Wright Patterson Laboratory, by the group of Rajesh Naik. Our work on the dynamic light scattering of coiled-coil-elastin block copolymers has been the critical component in determining the sizes of the self-assembled structures.